論　文　概　要

論 文 題 目 :

A study on a ligand for an inhibitory immunoreceptor, Allergin-1（抑制性免疫受容体のリガンドに関する研究）

指導教員：渋谷 和子

人間総合科学研究科 生命システム医学専攻
教授

所　　属：筑波大学大学院人間総合科学研究科
生命システム医学専攻

氏　　名：Almeida, Mariana Silva

目　　的：Type I hypersensitivity is an allergic reaction mediated by allergen-triggered generation of immunoglobulin E (IgE) antibodies. IgE binds to high affinity receptor for IgE (FcεRI) on mast cells (MCs), and induces the degranulation and release of inflammatory mediators by MCs. Despite the increasing understanding of type I allergic reactions, the development of new therapies is required for the better treatment and managements of allergic disorders, including anaphylaxis, asthma and food allergy. Allergy inhibitory receptor 1 (Allergin-1) is a membrane-type receptor, composed of two immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic region, and is expressed on human and murine myeloid cells. Allergin-1 on MCs suppress IgE-dependent FcεRI receptor signaling and inhibits systemic and cutaneous anaphylaxis and airways hyperesponsiveness. Furthermore, Allergin-1 on lung dendritic cells (DCs) recruits tyrosine phosphatase SHP-1 upon house dust mite (HDM) challenge to inhibit MyD88-dependent signaling and allergic airway inflammation. Allergin-1 on DCs becomes phosphorylated within minutes after HDM stimulation, however Allergin-1 ligand haven't been identified. The purpose of this study is to identify and characterize a ligand for Allergin-1.

対象と方法：To investigate the binding of Allergin-1 to its ligand, mouse Allergin-1 (msAlg) recombinant proteins was generated by tagging three FLAG epitopes in msAlg sequence (msAlg 3×FLAG). Expression of ligand was detected by incubation of bone marrow-derived cultured dendritic cells (BMDCs) with msAlg 3×FLAG protein and analyzed by flow cytometric analysis (FACS). Allergin-1-mediated signaling was monitored by mouse Allergin-1/NFAT/Ba/F3 reporter cell line. Allergin-1 reporter cells monitor specific ligand-binding through FcRγ-induced NFAT signaling pathway and expression of green fluorescence protein (GFP). Allergin-1-interacting molecules on BMDCs were isolated by immunoprecipitation and analyzed by liquid chromatography-mass spectrometry (LC-MS). Extracellular vesicles (EVs) from LPS-treated (mature) and naive (immature) BMDCs were isolated with EVs capture beads. Functional analysis was performed by incubation of WT and *Milr1*$^{-/-}$ IgE-sensitized bone marrow-derived mast cells (BMMCs) in the presence or absence of the prospective ligand and measuring BMMCs degranulation levels by ß-hexosaminidase assay.

結　　果：Flow cytometric analysis (FACS) showed that msAlg 3×FLAG protein bound to BMDCs. Stimulation of Allergin-1 reporter cells with BMDCs and BMDC-derived culture supernatant induced GFP expression, suggesting that BMDCs express and secrete an Allergin-1 ligand. Immunoprecipitation of Allergin-1 and its interacting molecules from BMDCs revealed six proteins, and database analysis demonstrated that they localize in exosomes. FACS analysis demonstrated that msAlg 3×FLAG protein bound to BMDC-derived EVs. Additionally, EVs-loading beads induced GFP expression by Allergin-1 reporter cells, suggesting that an

Allergin-1 functional ligand is expressed on BMDC-derived EVs. Immunoblotting of EVs with msAlg 3×FLAG detected binding to protein bands of approximately 36-38kDa. MS-LC analysis of 36-38kDa protein bands revealed Annexins A1-A5. Among them, only Annexin A5 induced GFP expression in Allergin-1 reporter cells. The expression of Annexin A5 on BMDC-derived EVs was detected by FACS and was increased upon LPS stimulation of BMDCs. Direct interaction between Annexin A5 and msAlg 3×FLAG protein in the presence of phosphatidylserine (PS) was detected by ELISA. Finally, in vitro analysis demonstrated that administration of Annxin A5 significantly inhibited IgE-dependent degranulation by WT BMMC, but not *Milr1*$^{-/-}$ BMMC, as compared to untreated controls. Thus, Annexin A5 is a ligand for Allergin-1.

考　　察: Direct interaction of Allergin-1 to Annexin A5 required the presence of plate-coated PS. Annexin A5 tridimensional structure goes through conformational change into an 'open form' in the presence of Ca^{2+} or PS in a Ca-dependent manner. Therefore, such structural change of Annexin A5 may be required for the binding to Allergin-1. The regulatory role of Annexin A5 in type I allergic disorders remains unknown. Further studies are required to characterize Annexin A5 in Allergin-1-mdeiated suppression of type I allergic reactions.

結　　論: This study has identified that Annexin A5 is a ligand for Allergin-1.